STUDY PLAN AMENDMENT

INCIDENCE OF PINE WOOD NEMATODES IN BARK BEETLE ATTACKED TREES[1]

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[1] Study listed on page 20 of Problem Analysis FS-SO-2203-3.0

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INTRODUCTION

The pine wood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle (= *B. lignicolus* Mamiya and Kiyohara) is either directly or indirectly the causative agent of pine wilt disease. Disease symptoms may be the result of phytotoxins produced by the nematode (Dropkin, et al. 1981, Bolla, Shaheen and Winter 1982), or a toxin produced by a bacterium, *Pseudomonas* sp., carried into the tree by the worm (Oku, et al. 1980). *B. xylophilus* utilizes fungus hyphae grown on wood tissue as its food (Kobayashi, Sasaki and Mamiya 1975). The fungi include species belonging to the genera *Ceratocystis, Pestalotis, Macrophoma, and Fusarium.*

In Japan, where pine wilt disease has been devastating forests for the past 30 years (Nickle 1981, Wingfield, et al. 1982a), the nematode is vectored by the Japanese sawyer beetle, *Monochamus alternatus* Hope. (Ishibashi and Kondo 1977). The life cycle of *B. xylophilus* is closely synchronized with that of its phoretic host and a truely remarkable mutualism exists among the sawyer beetle, PWN, and the various bacteria and fungi associated with them. The J_4 dauerlarva is transported to new hosts by entering the tracheae of callow adult beetles by way of the spiracles (Mamiya 1972, Togashi and Sekizuka 1982). The dauerlarvae of *B. xylophilus* and microorganisms are introduced into healthy trees through wounds made during maturation feeding of the beetle, and the resulting pine wilt disease stresses or kills the tree making it suitable for cerambycid oviposition and larval development (Dropkin, et al. 1981).

The pine wood nematode (= timber nematode) was originally found in Louisiana in 1931 and it is now believed that it was exported to Japan (Wingfield, et al 1982a). Damage caused by the pine wood nematode in the United states during the past 50 years was probably attributed to insects

and fungi (Nickle 1980). Native North American species such as long leaf pine (*Pinus palustris* Mill.), loblolly pine (*P. taeda* L.) and shortleaf pine (*P. echinata* Mill.) are partially resistant whereas many exotic species are susceptable to pine wilt disease (Futai and Furuno 1979, Mamiya 1976). The pine wood nematode is known to be present in 32 states and has been collected from 20 species of pine as well as from Atlantic cedar (*Cedrus atlantica* Manetti), deodar cedar (*C. deodara* (Roxb. Loud.)), European larch (*Larix decidua* Mill.), tamarack ((*L. laricina* Du Roi) K. Koch), and white spruce (*Picea glauca* Moench. Voss) (Dropkin, et al. 1981, Wingfield, et al. 1982a).

Pine wilt disease, in general, is endemic in the United States and not, at present, epidemic (Dropkin, et al. 1981) except in limited areas on introduced species (Adams and Morehart 1982). Damage is usually restricted to individual trees or plantations (Nickle 1982). However, the presence of pine wilt disease in the United States, especially in the southern states, has caused concern regarding the potential that this disease could inflict over the extensive pine stands now existing in the south (Anon. 1982, Dropkin, et al. 1981). Should this disease become epidemic in the United States, there is no known effective control. In Japan, pesticides are being applied to control the cerambycid vector, with limited success.

OBJECTIVES

The recent discovery of the pine wood nematode in the United States raises a number of questions regarding the interrelationships among nematodes, pine wilt disease, cerambycids and scolytids. Wingfield, et al. (1982b) only found *B. xylophilus* in trees colonized by suppossed secondary insects such as bark beetles and borers. They speculated that the pine wood nematode may only become established in stressed trees. However, scolytids and cerambycids

usually do not attack healthy pines and oviposit only in weakened or dead trees (Morimoto and Iwasaki 1972). Scolytids are not known to vector the pine wood nematode (Mamiya 1972, Kakuliya 1966). Therefore, the possibility exists that bark and engraver beetles may exploit a resource provided by cerambycids and the pine wood nematode. This study will determine the incidence of pine wilt disease in scolytid infestations.

METHODS AND MATERIALS

Trees determined to be the original tree attacked in a bark beetle infestation will be sampled for pine wood nematodes. Xylem samples will be taken from the bole by removing the outer bark, swabbing the exposed xylem with 70% ethanol, and drilling one inch deep into the xylem using a 3/4" wood bit. Samples will be taken from several portions of the bole and wood cuttings caught in Ziploc® sandwich bags, and transported to the laboratory in an ice chest. In the laboratory, some of the woodcuttings will be wrapped in Kimwipes® and soaked for 24 hours in distilled water. The water will then be examined for nematodes and any found transferred to a drop of 3% formalin on a glass slide and identified under a compound microscope. Additional xylem samples will be transferred to petri dishes containing the fungus *Monilinia fructicola* (Winter) Honey cultured on potato dextrose agar. After two weeks of incubation at 25°C. the petri dishes will be examined for nematodes and those found transferred to temporary microscope mounts and identified.

Data taken from stands in which trees have been sampled for PWN will include: forest type, stand age, site index, and hazard class. Independence of pine species and the incidence of PWN will be tested using Chi-square tests, and forest type, stand age, etc. of diseased and non-diseased trees will be compared using t-tests.

MATERIALS AND METHODS

Adult sawyer beetles (longhorn beetles) are attracted to dead and dying pines on which they mate and eventually deposit eggs in crater-like cavities chewed through the outer bark. Pine wood nematodes can be introduced into dead trees through these oviposition wounds just as they can be introduced into healthy trees through feeding wounds made by the beetle. The presence of these nematodes in southern pine products, especially wood chips, has recently become a problem to exporting companies.

To determine which species of sawyer beetles vector this pathogenic worm and to determine when during the year the nematode is vectored in greatest numbers, sawyer beetles flying to host trees will be trapped and examined for nematodes. Window traps baited with Ips attractant will be placed near areas where sawyer beetle populations are expected to exist. At least one species of sawyer beetle is known to be attracted to Ips attractant (Billings and Cameron, 1984). Since, as far as is known, trees used for maturation feeding are selected randomly by the beetle, and no feeding attractant is known, it is not possible to trap recently emerged beetles.

APPLICATION OF RESEARCH RESULTS

Results of this study will be presented in a final report consisting of a manuscript or published paper. This study should provide data on which to base a decision for conducting future research on the susceptibility of southern pines to pine wilt disease.

SAFETY AND HEALTH CONSIDERATIONS

This study presents minimal risks to the safety and health of the investigators. No extraordinary safety measures need to be adressed other than safe work practices standard to the laboratory environment.

ENVIRONMENTAL CONSIDERATIONS

The research activities described here are limited in scope and will have no impact in terms of changes in the physical, biological, economic, or social components of the environment thereby qualifying as a categorical exclusion (FSM-1952).

PERSONNEL ASSIGNMENT

Donald N. Kinn	Research Entomologist
William J. Oakes	Biological Technician

This study will be initiated in 1983 and is expected to cover a period of three years, terminating in 1986.

ESTIMATED EXPENSES

Labor (wages)	$15,000
Travel.....	$4,000
Equipment	
Nonexpendable	
Microscope.....	$3,000

Expendable

Glassware	$2,000
Culture media	$1,000
Hand tools	$500
Misc. expenses	$3,000

COOPERATIVE RESEARCH

Field data (site, stand and tree characteristics) required for another study (FS-SO-2203-6.15) will be collected by Erich G. Vallery and temporary employees under the supervision of Peter L. Lorio, Jr. and Robert A. Sommers. Xylem samples will be taken from trees in selected plots when feasible.

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